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(54) Title: NOVEL DERIVATIVES OF SWAINSONINE, PROCESSES FOR THEIR PREPARATION, AND THEIR USE AS THERAPEUTIC AGENTS

(57) Abstract

The invention relates to 8a substituted swainsonine derivatives, processes for their preparation, and their use as therapeutic agents. The invention also relates to pharmaceutical compositions containing the compounds and their use as therapeutics.

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Title: NOVEL DERIVATIVES OF SWAINSONINE, PROCESSES FOR THEIR PREPARATION, AND THEIR USE AS THERAPEUTIC AGENTS

FIELD OF THE INVENTION

The present invention relates to novel 8a substituted derivatives of swainsonine, processes for their preparation, and their use as therapeutic agents.

BACKGROUND OF THE INVENTION

Carbohydrate structures present on human tumor cells have been associated with cancer invasion and metastasis (Dennis *et. al.*, *Science 236*: 582, 1987; Demetriou *et. al.*, *J. Cell Biol. 130*:383, 1995). These structures include the GlcNAc β(T1-6) branched *N*- and *O*-linked carbohydrate side chains of cell-surface glycoproteins. The Golgi enzymes required for their synthesis are β(T1-6) *N*-acetylglucosaminyltransferase V (i.e. GlcNAc-TV) and core 2 β(T1-6) *N*-acetylglucosaminyltransferase (i.e. core 2 GlcNAc-T), respectively. These enzymes are up-regulated in human carcinomas (Fernandes et al., Cancer Res. 51:718-723, 1991), a phenomenon that has been associated with the activation of the *ras* signaling pathway (Dennis et al., Science 236:582-585, 1987; Dennis et al Oncogene 4:853-860, 1989)). Furthermore, overexpression of GlcNAc-TV in epithelial cells results in morphological transformation and tumor formation in mice (Demetriou et al, J. Cell Biol. 130:383-392, 1995). Therefore, GlcNAc-TV as well as enzymes supplying acceptor substrates to GlcNAc-TV (i.e. GlcNAc-TI, α-mannosidase II and core 2 GlcNAc-T of the *O*-linked pathway) are targets for anti-cancer pharmaceuticals.

A lead α-mannosidase II inhibitor, swainsonine, has been tested in preclinical and human trials. Swainsonine is an indolizidine alkaloid found in Australian *Swainsona canescens* (Colegate et al., Aust J Chem 32:2257-2264, 1979), North American plants of the genera *Astragalus* and *Oxytropis* (Molyneux RJ and James LF., Science 215:190-191, 1981), and also the fungus *Rhizoctonia leguminicola* (Schneider et al., Tetrahedron 39;29-31, 1983). Swainsonine's ability to inhibit α-mannosidase II activity appears to be responsible for its interesting immunomodulating and cancer suppression activity. Swainsonine is believed to function as an enzyme inhibitor because it can mimic the glycosylium cation intermediate generated during the hydrolytic cleavage of mannopyranosides. (Goss, P.E. et al., *Clin. Cancer Res. 1*: 935-944, 1995).

The swainsonine blockage of α-mannosidase II is prior to GlcNAc-TV and prevents expression of GlcNAc β(T1-6) branched *N*-linked carbohydrates. Swainsonine-treated murine tumor cells have been found to be less metastatic in both organ-colonization and spontaneous metastasis assays in mice (Dennis J.W., Cancer Res. 46:5131-5136, 1986 and Humphries et al., Proc. Natl. Acad. Sci. USA 83:1752-1756, 1986). Swainsonine has also been shown to block tumor cell invasion through extracellular matrix *in vitro* (Yegel et al., Int. J. Cancer 44:685-690,

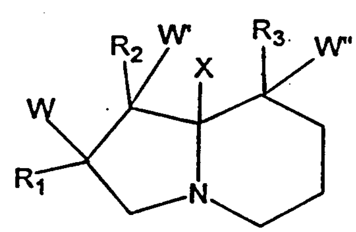
1989 and Seftor et al., Melanoma Res. 1:53-54, 1991). Swainsonine administered either orally or by mini-osmotic pumps to athymic nude mice inhibited the growth rate of human MeWo melanoma and HT29m colon carcinoma tumor xenografts in the mice (Dennis et al., J. Natl. Cancer Inst. 81:1028-1033, 1989 and Dennis et al., Cancer Res., 50:1867-1872,1990). Phase 1 clinical trials of swainsonine have been done which indicate that it has efficacy in the treatment of human tumors. (Goss *et. al*, *Cancer Res.*, *54*:1450, 1995).

Swainsonine has positive effects on cellular immunity in mice (reviewed in Humphries M.J. and Olden K., Pharmacol Ther. 44:85-105, 1989, and Olden et al., Pharmacol Ther 50:285-290, 1991)). In particular, swainsonine has been shown to alleviate both chemically-induced and tumor-associated immune suppression (Hino et al., J. Antibiot. (Tokyo) 38:926-935, 1985), increase NK cell (Humphries et al., Cancer Res. 48:1410-1415, 1988), and LAK cell activities (Yagita M and Saksela E., Scand. J. Immunol. 31:275-282, 1990), and increase splenic and bone marrow (BM) cell proliferation (White et al., Biochem. Biophys. Res. Commun. 150;615-625, 1988; Bowlin et al. Cancer Res 49, 4109-4113, 1989, and White et al., Cancer Commun. 3:83-91, 1991). SW has also been shown to be hemorestorative in mice following treatment with both cycle-specific and nonspecific chemotherapeutic agents (Oredipe et al., J. Natl. Cancer Inst. 83:1149-1156, 1991).

Selected indolizidine derivatives and swainsonine analogues have been reported in the literature (Dennis, J. W. et al. Biochemical Pharmacology 46:1459-1466, Japanese Patent Application No. J61277685, U.S Patent No. 5,466,809, U.S Patent No 5,650,413, WO96/40683, WO98/14446, and WO98/14445).

SUMMARY OF THE INVENTION

The present invention relates to a compound of the formula I



I

wherein

(a) R_1, R_2 and R_3 are each independently hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, or R_1 and R_2 together form a carbocyclic or heterocylic ring;

(b) W and W" and W' are each independently hydrogen, hydroxyl, alkoxy, thiol, thioalkyl, thioaryl, halo or amino, or W and W' together form a carbocyclic or heterocyclic ring; and

(c) X represents alkyl, alkenyl, alkynyl, cycloalkyl, alkoxy, or aryl,

and salts and optically active and racemic forms of a compound of the formula I.

The present invention also provides a process for the preparation of a compound of the formula I as defined herein.

The compounds of the invention have valuable pharmacological properties and they provide antimicrobial, cancer suppressing effects, hemorestorative, chemoprotective, radioprotective, and immunomodulatory properties. Therefore, the invention contemplates a pharmaceutical composition comprising a compound of the formula I as an active agent.

The invention further relates to a method for stimulating the immune system, stimulating hematopoietic progenitor cell growth, treating proliferative disorders or microbial or parasitic infections, or conferring protection against chemotherapy and radiation therapy in a patient comprising administering an effective amount of a compound of the formula I of the invention. The invention also relates to the use of a compound of the formula I in the preparation of a medicament for stimulating the immune system, stimulating hematopoietic progenitor cell growth, or conferring protection against chemotherapy and radiation therapy, and/or for treating proliferative disorders, and microbial or parasitic infections.

The present invention also relates to the use of a compound of the formula I which is esterified at free hydroxyls as a prodrug.

These and other aspects of the present invention will become evident upon reference to the following detailed description and attached drawing.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention will now be described in relation to the drawings in which:

Figure 1 is a schematic diagram of a reaction for preparing a compound of the invention.

DETAILED DESCRIPTION OF THE INVENTION

I. COMPOUNDS OF THE INVENTION

Hereinabove and in the following the term "alkyl", alone or in combination, refers to a branched or linear hydrocarbon radical, typically containing from 1 through 20 carbon atoms, preferably 1 through 10 carbon atoms, more preferably 1 to 6 carbon atoms. Typical alkyl groups include but are not limited to methyl, ethyl, 1-propyl, 2-propyl, 1-butyl, 2-butyl, tert-butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, and the like.

The term "alkenyl", alone or in combination, refers to an unsaturated branched or linear group typically having from 2 to 20 carbon atoms and at least one double bond. Examples of such groups include but are not limited to ethenyl, 1-propenyl, 2-propenyl, 1-butenyl, 1,3-butadienyl, 1-hexenyl, 2-hexenyl, 1-pentenyl, 2-pentenyl, and the like.

The term "alkynyl", alone or in combination, refers to an unsaturated branched or linear group having 2 to 20 carbon atoms and at least one triple bond. Examples of such groups include but are not limited to ethynyl, 1-propynyl, 2-propynyl, 1-butynyl, 2-butynyl, 1-pentynyl, and the like.

The term "cycloalkyl" refers to cyclic hydrocarbon groups and includes but is not limited to cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, and cyclooctyl.

The term "aryl", alone or in combination, refers to a monocyclic or polycyclic group, preferably a monocyclic or bicyclic group. An aryl group may optionally be substituted as described herein. Examples of aryl groups and substituted aryl groups are phenyl, benzyl, p-nitrobenzyl, p-methoxybenzyl, biphenyl, and naphthyl.

The term "alkoxy" alone or in combination, refers to an alkyl or cycloalkyl linked to the parent molecular moiety through an oxygen atom. Examples of alkoxy groups are methoxy, ethoxy, propoxy, vinyloxy, allyloxy, butoxy, pentoxy, hexoxy, cyclopentoxy, cyclohexoxy, and the like.

The term "halo" or "halogen", alone or in combination, means fluoro, chloro, bromo, or iodo.

The term "amino", alone or in combination, refers to a chemical functional group where a nitrogen atom (N) is bonded to three substituents being any combination of hydrogen, alkyl, cycloalkyl, alkenyl, alkynyl, or aryl with the general chemical formula $-NR_4R_5$ where R_4 and R_5 can be any combination of hydrogen, alkyl, cycloalkyl, alkenyl, alkynyl, or aryl. Optionally one substituent on the nitrogen atom can be a hydroxyl group (-OH) to give an amine known as a hydroxylamine. Examples of amino groups are amino ($-NH_2$), methylamine, ethylamine, dimethylamine, 2-propylamine, butylamine, isobutylamine, cyclopropylamine, benzylamine, allylamine, hydroxylamine, cyclohexylamino ($-NHCH(CH_2)_5$), piperidine ($-N(CH_2)_5$) and benzylamino ($-NHCH_2C_6H_5$).

The term "thioalkyl", alone or in combination, refers to a chemical functional group where a sulfur atom (S) is bonded to an alkyl. Examples of thioalkyl groups are thiomethyl, thioethyl, and thiopropyl.

The term "thioaryl", alone or in combination, refers to a chemical functional group where a sulfur atom (S) is bonded to an aryl group with the general chemical formula $-SR_6$ where R_6 is an aryl group which may be substituted. Examples of thioaryl groups and substituted thioaryl groups are thiophenyl, para-chlorothiophenyl, thiobenzyl, 4-methoxy-thiophenyl, 4-nitro-thiophenyl, and para-nitrothiobenzyl.

The term "carbocyclic" refers to molecular rings where the framework is constructed by joining carbon atoms solely and includes but is not limited to any stable 3- to 7- membered monocyclic or bicyclic or 7- to 14-membered bicyclic or tricyclic or up to 26-membered polycyclic carbon ring, any of which may be saturated, partially unsaturated, or aromatic. Examples of carbocyclic rings include substituted or unsubstituted cycloalkyl, monocyclic unsaturated hydrocarbons, and aryl as described herein, including but not limited to benzene and napthalene.

Heterocyclic rings are molecular rings where one or more carbon atoms have been

replaced by hetero atoms (atoms not being carbon) such as for example, oxygen (O), nitrogen (N) or sulfur (S), or combinations thereof. Examples of heterocyclic rings include ethylene oxide, tetrahydrofuran, thiophene, piperidine (piperidinyl group), pyridine (pyridinyl group), and caprolactam. A carbocyclic or heterocyclic group may be optionally substituted at carbon or nitrogen atoms with for example, alkyl, phenyl, benzyl or thienyl, or a carbon atom in the heterocyclic group together with an oxygen atom may form a carbonyl group, or a heterocyclic group may be fused with a phenyl group.

One or more of R_1, R_2, R_3, W, W', W", and/or X, alone or together, which contain available functional groups as described herein, may be substituted with for example one or more of the following: alkyl, alkoxy, hydroxyl, aryl, cycloalkyl, alkenyl, alkynyl, thiol, thioalkyl, thioaryl, amino, or halo. The term "one or more" used herein preferably refers to from 1 to 3 substituents, most preferably 1 to 2 substituents.

In an embodiment of the invention, compounds of the formula I are provided where R_1, R_2, and R_3, are the same and represent hydrogen; W, W', and W" are the same and represent hydroxyl; R_1, R_2, and R_3 are the same or different and represent alkyl, alkenyl, alkynyl, or aryl, preferably alkyl or aryl, most preferably alkyl; R_1 and R_2 together form a carbocyclic or heterocyclic ring; W, W', and W" represent hydroxyl, alkoxy, thiol, thioalkyl, thioaryl, halo, or amino; W and W' together form a carbocyclic or heterocyclic ring; or X represents alkyl, alkenyl, alkynyl, aryl, cycloalkyl or alkoxy, preferably alkyl or alkoxy, more preferably alkyl.

Preferred compounds of the formula I of the invention are those where R_1, R_2, and R_3 represent hydrogen, and W, W', and W" represent hydroxyl, and X represents methyl, ethyl, phenyl, benzyl, or methoxy.

Particularly preferred compounds of the invention are (1*S*, 2*R*, 8*R*, 8a*R*)-8a-methyl-1,2,8-trihydroxyindolizidine, (1*S*, 2*R*,8*R*, 8a*R*)-8a-ethyl-1,2,8-trihydroxy-indolizidine, (1*S*, 2*R*, 8*R*, 8a*R*)-8a-propyl-1, 2, 8-trihydroxyindolizidine, and (1*S*, 2*R*, 8*R*, 8a*R*)-8a-butyl-1, 2, 8-trihydroxyindolizidine.

It will be appreciated that, owing to the asymmetrically substituted carbon atoms in formula I, a compound of formula I may exist in, and be isolated in, optically active and racemic forms. It is to be understood that the present invention encompasses a compound of formula I as a mixture of diastereomers, as well as in the form of an individual diastereomer, and that the present invention encompasses a compound of formula I as a mixture of enantiomers, as well as in the form of an individual enantiomer. It will be appreciated that the enantiomers and diastereomers are convertible by facile epimerization of the chiral centers, and that a preparation containing a compound of formula I as a mixture of isomers of the formula I is within the scope of the invention.

Therefore, the present invention contemplates all optical isomers and racemic forms thereof of the compounds of the invention, and the formulas of the compounds shown herein are

intended to encompass all possible optical isomers of the compounds so depicted.

The present invention also contemplates salts and esters of the compounds of the formula I of the invention. In particular, the present invention includes pharmaceutically acceptable salts. By pharmaceutically acceptable salts is meant those salts which are suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response and the like, and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well known in the art and are described for example, in S. M. Berge, et al., J. Pharmaceutical Sciences, 1977, 66:1-19. Illustrative of such salts are the salts with inorganic acids such as, for example, hydrochloric, hydrobromic, sulfuric, phosphoric, and like acids; with organic carboxylic acids, such as for example acetic, propionic, glycolic, lactic, pyruvic, malonic, succinic, fumaric, malic, tartaric, citric, ascorbic, maleic, hydroxymaleic, and dihydroxymaleic, benzoic, phenylacetic, 4-aminobenzoic, 4-hydroxybenzoic, anthranilic, cinnamic, salicylic, 4-aminosalicylic, 2-phenoxybenzoic, 2-acetoxybenzoic, mandelic, and like acids; and with organic sulfonic acids such as methanesulfonic acid, and p-toluenesulfonic acid. Such salts can be obtained by standard procedures from an amine of this invention and the appropriate acid.

Crystalline forms of the compounds of the formula I of the invention are also contemplated.

II. PROCESSES FOR PREPARING COMPOUNDS

The compounds of the formula I of the present invention can be prepared by utilizing procedures and techniques well known and appreciated by one of ordinary skill in the art. By way of illustration, descriptions of some methods that may be used to prepare compounds of the formula I of the invention are set forth herein.

Compounds of the Formula I may be synthesized in a variety of ways by adapting common synthetic organic chemistry practices to known synthetic intermediates. For example, compounds of the formula I where X is alkyl may be synthesized by protecting swainsonine acetonide (1) at the 8 position with a protecting group; converting the protected swainsonine acetonide to an N-oxide; reacting the N-oxide with trifluoroacetic anhydride; reacting the acylated N-oxide with a nucleophile under basic conditions for example, an alkyl magnesium bromide; and removing the protecting groups.

In an embodiment of the invention, (1*S*, 2*R*, 8*R*, 8a*R*)-8a-methyl-1, 2, 8-trihydroxyindolizidine is prepared by converting swainsonne acetonide to (1*S*, 2*R*, 8*R*, 8a*R*)-8-methoxymethoyl-1,2-(isopropylidenedioxy)indolizidine by adding tetrabutyl-ammonium iodide, methoxy methyl chloride, and sodium hydride in THF; reacting (1*S*, 2*R*, 8*R*, 8a*R*)-8-methoxymethoxy-1, 2-(isopropylidenedioxy)indolizidine with hydrogen peroxide in dichloromethane-ethanol to yield (1*S*, 2*R*, 8*R*, 8a*R*)-8-methoxymethoxy-1,2-(isopropylidenedioxy)indolizidine *N*-oxide (3); reacting the N-oxide with trifluoroacetic anhydride followed by an alkylmagnesium bromide to yield (1*S*, 2*R*, 8*R*, 8a*R*)-8-methoxymethoxy-8a-alkyl-

1, 2-(isopropylidenedioxy)-indolizidine (4); and removing the protecting groups to yield (1*S*, 2*R*, 8*R*, 8a*R*)-8a-methyl-1, 2, 8-trihydroxyindolizidine. This reaction is illustrated in the scheme shown in Figure 1.

Reactive groups used in processes for preparing the compounds of the invention may be blocked using appropriate protective groups. Appropriate blocking and deblocking schemes are known to the skilled artisan (See T.W. Greene, Protective Groups in Organic Synthesis, 2nd Ed, T.W. Greene, and P.G.M. Wats, John Wiley & Sons, New York, 1991). In general, particular, protective groups are selected which adequately protect the reactive groups in question during subsequent synthetic steps and which are readily removable under conditions which will not cause degradation of the desired product. By way of example, ethers, silyl ethers, orthoesters, acetals, ketals, and esters can be used to protect isolated hydroxyl groups. In particular, suitable protective groups which may be used in the process of the invention include O-benzyl, O-para-methoxybenzyl, O-acetoxy, O-benzoyloxy, O-pivaloyl, O-allyl, methoxymethyl, isopropylidene, benzylidene, methylidene, acetylidene, 1-methoxy ethylidene, 1,3-(1,1,3,3)-tetraisopropyldisiloxanylidene, O-trimethyl silyl, O-t-butyl dimethylsilyl. Removal of the protective groups may be carried out using procedures known in the art.

Methods or references to methods for transformation of groups such as hydroxyl to groups such as halo, amino, or alkoxy, with or without inversion of configuration are known to those skilled in the art and can be found in for example "Organic Functional Group Preparations" 2nd Ed., S.R. Sandler and W. Kare, Academic Press; "Comprehensive Organic Transformations", R.C. Larock, VCH Publishers, 1989, "Advanced Organic Chemistry" 4th Ed. By J. March, Wiley Interscience, 1992; and "Compendium of Organic Synthetic Methods" John Wiley & Sons. Representative methods are described herein.

A free hydroxyl group may be converted to an alkoxy group in blocked/deblocked compounds to produce compounds where for example one or more of W, W', and W" are alkoxy, by reacting with an alkyl halide in the presence of a base.

An alkoxy group may be added by dissolving a compound with a free hydroxyl in DMF and adding it to a flask under an inert atmosphere containing a base (e.g. sodium hydride) at low temperature (0°C to 10°C). After stirring for a few minutes, benzyl bromide in DMF is added dropwise at low temperature, for example 0°C to 10°C. The reaction mixture is further stirred at room temperature for 2 to 24 hours.

A halo group, for example, fluoro, may be added by dissolving a compound with a free hydroxyl in dichloromethane (DCM) together with a base like pyridine. After cooling at low temperature (-10°C to -60°C), an appropriate amount of triflic anhydride, or mesyl chloride, or tosyl chloride is added dropwise. The reaction is allowed to stir at a temperature between 0°C to 25°C. Conventional work-up of the reaction mixture yields the esterified compound. Treatment of this derivative with sodium benzoate in DMF is carried out immediately, which replaces the

leaving group with O-benzoate with inversion of configuration at the carbon center. The free hydroxyl is generated by treatment with a base (e.g. sodium methoxide) and then reblocked by a suitable leaving group such as triflate. To obtain a fluoro derivative with inversion, the triflate is treated with anhydrous tetra alkyl ammonium fluorides (preferably tetra n-butyl) or potassium fluoride in a suitable solvent (e.g diethyl ether, tetrahydrofuran or crown ether). Alternatively, the hydroxyl group is simply treated with (diethyl amino) sulfur trifluoride (DAST) in a one-step fluorination reaction.

For the introduction of an amino group, the triflate may be treated either with sodium azide, phthalimide, or benzyl amine in DMF. The product may be obtained with an azido or benzyl amine group, with inversion, which on reduction with palladium on carbon in a hydrogen atmosphere (azide and benzylamine), or treatment with hydrazine or methyl amine (phthalimido) gives the free amino group.

Appropriate methods for introducing a thiol group in compounds of the formula I are well known to the skilled artisan. For example, a thiol group may be added by nucleophilic substitution of an alkyl halide or sulfonyl ester for example using sodium sulfhydride (NaSH) or, by nucleophilic substitution of a halide or sulfonate ester using thioacetic acid to give a thioacetate group which can then be deblocked to a free thiol upon treatment with sodium methoxide in methanol by converting the same to a Bunte salt using thiosulfate ($S_2O_3^{2-}$) and later hydrolyzing the Bunte salt with an acid or, by treating the hydroxyl group with a fluoropyridinium salt and N,N-dimethyl thiocarbamate (Hojo: Yoshino: Mukaiyama Chem. Lett. (1977) 133:437) or, by oxidizing a hydroxyl to a ketone then converting it to a thioketone with Lawson's reagent and reducing to a thiol with sodium borohydride. For a review, see (Wardell, in Patai "The Chemistry of the Thiol Group, pt 1: Wiley: New York, 1974, pp. 179-211).

Methods for introducing a thioalkyl or a thioaryl group in a compound of the formula I are also well known to the skilled artisan. For example, by nucleophilic substitution of an alkyl halide or sulfonyl ester for example with alkyl or aryl thiolate salts or with alkyl or aryl thiols in the presence of a base such as 1,8-diazabicyclo[5.4.0] undecene (DBU), by alkylating thiols with alkyl or aryl halides or sulfonate esters or, by treating a hydroxyl group with an alkyl or aryl halide in the presence of tetramethyl thiourea followed by sodium hydride (Fujisaka; Fujiwara; Norisue; Kajigaeshi Bull. Chem. Soc. Jpn. 1985, 58:2529) or, by treating an alcohol with tributyl phosphine and an N-(thioaryl)succinimide in benzene (Waters Tetrahedron Lett. 1977, p. 4475 and references cited within). For a review, see Peach, in Patai "The Chemistry of the Thiol Group, pt 1: Wiley: New York, 1974, pp 721-735.

In addition, appropriate methods for replacing a blocked or deblocked hydroxyl group with a hydrogen in compounds of the formula I are well known to the skilled artisan. For example, alkyl halides or sulfonyl esters such as tosylates can be selectively reduced with lithium aluminum hydride or a variety of other metal hydride reducing agents in different solvents such as ether. A

large list of methods able to achieve this transformation is provided in J. March "Advanced Organic Chemistry. Reactions, Mechanisms and Structure" 4th Edition, 1992, pp 438- 446 and references cited therein.

Some alkyl or aryl groups, particularly those which may contain unsaturations or other chemical functional groups such as hydroxyl, or alkoxy for example, can be further derivatized by chemical processes such as oxidation, hydroxylation, hydrolysis, alkylation, reduction, carbon-carbon chain elongation by Grignard or Wittig reactions for example to introduce new or additional functional groups in any final compound. Such transformations can be achieved by anyone skilled in the art of synthetic organic chemistry.

If necessary, the products of the processes described above may be purified by conventional methods such as column chromatography.

Compounds of the formula I with available hydroxyl groups can be converted to epi-isomers by SN_2 inversion. For example, a free hydroxyl may be reacted with mesyl chloride and pyridine to give O-mesyl (methyl sulphonyl), which on treatment with sodium benzoate in DMF (dimethyl formamide) produces a compound where the free hydroxyl group is replaced by epi-O-benzoate. Alternatively, a Mitsunobu reaction can be used to provide the epibenzoate (O. Mitsunobu, Synthesis, January 1981, p. 1-28). Deesterification using NaOMe in methanol results in a compound of the formula I where the free hydroxyl is replaced by epihydroxyl.

The compounds of the formula I described above may be converted into salts using conventional procedures.

Compounds of the formula I with free hydroxyl groups may also be converted into esters using conventional procedures. For example, a compound of the formula I may be dissolved in DCM and pyridine. After cooling (0°C to 5°C) benzoic anhydride or benzoyl chloride in DCM and pyridine is added dropwise. The reaction is allowed to stir at room temperature for 2 to 24 hours. Conventional work-up yields the esterified derivatives.

Optical antipodes of the compounds of the formula I may be prepared from the corresponding racemic forms by standard resolution techniques, involving, for example, the separation of diastereomeric salts of those compounds of the formula I characterized by the presence of a basic amino group, and an optically active acid, or by synthesis from optically active precursors.

III. UTILITY OF COMPOUNDS OF THE INVENTION

The compounds of the formula I are inhibitors of oligosaccharide processing and in particular are inhibitors of mannosidase. General mannosidase inhibition may be tested by measuring the inhibition of Jack Bean, α-mannosidase, or lysosomal α-mannosidase. Mannosidase inhibition may also be tested using an L-PHA toxicity assay. The assay is based on the finding that the specific binding of the toxic plant lectin L-PHA to transformed cell lines such as MDAY-D2 tumor cells is a specific measure of inhibition of oligosaccharide processing. The

measurement of IC_{50} in the L-PHA toxicity assay reflects the ability of the compound to enter into cells and to effect inhibition of oligosaccharide processing. It is a general screen for activity in cells which measures cell entry, inhibition of the target enzyme, α-mannosidase II in the Golgi, and the resulting cellular phenotype.

Therefore, a compound of the invention may be tested for its ability to inhibit N-linked oligosaccharide processing by growing transformed cells in the presence of L-PHA and the compound; measuring the amount of proliferation of the cells; and determining the ability of the compound to inhibit N-linked oligosaccharide processing by comparing the amount of proliferation of the cells with the amount of proliferation observed for the cells grown in the presence of L-PHA alone.

Transformed cells which may be used in this assay include MDAY-D2, L1210, CHO, B16, melanoma tumor cells, and human tumor cells such as SW 480, LS174T, HT-29, WiDr, T2, MDA-231, MCF7, BT-20, Hs578T, K562, Hs578T, SK-BR-3, CY 6T, MDA-468, H23, H157, H358, H1334, H1155, H28, H460, Hmesol, H187, H510A, N417, H146, H1092, H82 (Restifo, N. P. et al, J. Exper. Med. 177:265-272, 1993).

The amount of proliferation of the cells may be measured using conventional techniques. For example, cell proliferation may be measured by measuring incorporation of labeled thymidine. More particularly, radioactively labeled thymidine may be added for about 2-5 hours, preferably 3-4 hours and the cells can be harvested and radioactivity counted using a scintillation counter.

The conditions for carrying out the above assay will be selected having regard to the nature of the compound and the cells employed. For example, if the transformed cells are MDAY-D2 tumor cells a concentration of about 1-4 x 10^4 cells, preferably 2 x 10^4 may be used. The MDAY-D2 cells are generally cultured for about 10 to 30 hours, preferably 18 to 20 hours, followed by addition of L-PHA at a concentration of about 10-50 μg/ml, preferably 20-30 μ/ml, more preferably 25 μg/ml.

The following L-PHA assay may be used to assay for inhibition of oligosaccharide processing (i.e. Golgi α-mannosidase II) in viable cells. MDAY-D2 tumor cells are inoculated into 96 well micro-test plates at 2 x 10^4 cells/well, containing serial dilutions of the compound to be tested in MEM plus 10% FCS. The cells are cultured for 18-20 hours, followed by the addition of L-PHA at 25 μg/ml for an additional 24 hours. Cell proliferation is measured by adding 0.5 μCi/well of ^{3}H-thymidine for 3-4 hours, harvesting onto glass fibre disks using a Titertek harvester, and counting the disks in a liquid scintillation counter. The apparent IC_{50} values for the test compounds are the drug concentrations showing 50% protection from L-PHA toxicity; that is 50% ^{3}H-thymidine incorporated compared with cells grown in the absence of L-PHA.

The ability of the compounds of the formulae I in which the free hydroxyls have been esterified, to be converted into more active compounds in cells can be measured by performing the L-PHA toxicity assay in the presence of an esterase inhibitor such as diethyl p-nitrophenyl

phosphate. For example, the esterase inhibitor diethyl p-nitrophenyl phosphate can be added to MDAY-D2 cells in the above described assay method about 4 hours prior to the α-mannosidase inhibitors. An increase in IC_{50} in the L-PHA toxicity assay in the presence of diethyl p-nitrophenyl phosphate indicates that the compound requires activation by esterases and would accordingly be useful as a prodrug.

The compounds of the formula I have valuable pharmacological properties and they provide immunostimulatory, antimicrobial and anti-cancer effects. In particular, the compounds are useful in the prevention, treatment, and prophylaxis of tumor growth and metastasis of tumors. The anti-metastatic effects of the compounds of the invention may be demonstrated using a lung colonization assay. For example, melanoma cells treated with a compound may be injected into mice and the ability of the melanoma cells to colonize the lungs of the mice may be examined by counting tumor nodules on the lung after death. Suppression of tumor growth in mice by the compound administered orally or intravenously may be examined by measuring tumor volume. Examples of protocols for confirming the activities of the compounds of the invention are included in the Example section.

The compounds of the invention may be especially useful in the treatment of various forms of neoplasia such as leukemias, lymphomas, melanomas, adenomas, sarcomas, and carcinomas of solid tissues in patients. In particular the composition may be useful for treating malignant melanoma, pancreatic cancer, ovarian cancer, cervico-uterine cancer, cancer of the kidney, stomach, lung, rectum, breast, bowel, gastric, liver, thyroid, head and neck such as unresectable head and neck cancers, lymphangitis carcinamatosis, cervix, salivary gland, leg, tongue, lip, bile duct, pelvis, mediastinum, urethra, bronchogenic, bladder, esophagus and colon, non-small cell lung cancer, and Kaposi's Sarcoma which is a form of cancer associated with HIV-infected patients with Acquired Immune Deficiency Syndrome (AIDS). The compounds may also be used for other anti-proliferative conditions such as arthrosclerosis and viral infections, in particular AIDS or hepatitis C.

The compounds of the formula I may be used to stimulate bone marrow cell proliferation (hemorestoration), in particular following chemotherapy or radiotherapy. The myeloproliferative activity of a compound of the formula I may be determined by injecting the compound into mice, sacrificing the mice, removing bone marrow cells and measuring the ability of the compound to stimulate bone marrow proliferation by directly counting bone marrow cells and by measuring clonogenic progenitor cells in methylcellulose assays.

The compounds of the invention are immune modulators and in particular they have immunostimulatory properties. Therefore, the compounds of the formula I may be used in cases where a patient has been immunocompromised such as patients infected with HIV or hepatitis C, or other viruses or infectious agents including bacteria, fungi, and parasites, in patients undergoing bone marrow transplants, and in patients with chemical or tumor-induced immune suppression.

The compounds also have an antiviral effect in particular on membrane enveloped viruses such as retroviruses, influenza viruses, cytomegaloviruses and herpes viruses. The compounds of the invention may also be used in the treatment of inflammation. In particular, they may be useful in the treatment of arthritis and asthma. The compounds may render carbohydrate structures on neutrophils unable to bind selectins. Selectins present at the site of damage interact with the carbohydrate structures on neutrophils in such a way that the neutrophils roll along the epithelial wall, stick, infiltrate, and cause tissue damage.

The compounds of the invention may be used to protect against the lethality of various chemotherapeutic agents as well as against lethal doses of irradiation. The compounds may also be used in the prevention of tumor recurrence after surgery i.e. adjuvant therapy.

The term "patient" refers to a warm-blooded animal such as a mammal which is afflicted with a particular disease state or condition as described herein. Examples of animals within the scope of the meaning of the term are dogs, cats, rats, mice, horses, bovine cattle, sheep, and humans.

The compounds may be converted using customary methods into pharmaceutical compositions. The pharmaceutical compositions contain the compounds either alone or together with other active substances. Such pharmaceutical compositions can be for oral, topical, rectal, parenteral, local, inhalant, or intracerebral use. They are therefore in solid or semisolid form, for example, pills, tablets, creams, gelatin capsules, capsules, suppositories, soft gelatin capsules, liposomes (see for example, U.S. Patent Serial No. 5,376,452), gels, membranes, and tubelets. For parenteral and intracerebral uses, those forms for intramuscular or subcutaneous administration can be used, or forms for infusion or intravenous or intracerebral injection can be used, and can therefore be prepared as solutions of the compounds or as powders of the active compounds to be mixed with one or more pharmaceutically acceptable excipients or diluents, suitable for the aforesaid uses and with an osmolarity which is compatible with the physiological fluids. For local use, those preparations in the form of creams or ointments for topical use or in the form of sprays should be considered; for inhalant uses, preparations in the form of sprays, for example nose sprays, should be considered.

The pharmaceutical compositions can be prepared by per se known methods for the preparation of pharmaceutically acceptable compositions which can be administered to patients, and such that an effective quantity of the active substance is combined in a mixture with a pharmaceutically acceptable vehicle. Suitable vehicles are described, for example, in Remington's Pharmaceutical Sciences (Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pa., USA 1985). On this basis, the pharmaceutical compositions include, albeit not exclusively, the compounds in association with one or more pharmaceutically acceptable vehicles or diluents, and contained in buffered solutions with a suitable pH and iso-osmotic with the physiological fluids.

The compounds are indicated as therapeutic agents either alone or in conjunction with other therapeutic agents or other forms of treatment (e.g. chemotherapy or radiotherapy). The compounds of the invention may be administered concurrently, separately, or sequentially with other therapeutic agents or therapies.

In particular, the compounds of the invention may be used in combination with anti-proliferative agents, antimicrobial agents, immunostimulatory agents, or anti-inflammatories. For example, the compounds may be used in combination with anti-viral and/or anti-proliferative agents such as a Th1 cytokine. Th1 cytokines include interleukins-2 and 12 (IL-2, IL-12), and the interferons-α, β, γ (IFN-α, IFN-β, IFN-γ), and inducers thereof. The compounds of the invention can be used with poly (I.C.), poly (I.C.)-LC, tumor necrosis factor (TNF), or transforming growth factor (TGF). The compounds may be administered to a patient being treated with a myelosuppressive agent, or a bone marrow transplant recipient. The compounds can be used in combination with chemotherapeutic agents including doxorubicin, 5-fluorouracil, cyclophosphamide, and methotrexate, with isoniazid for the prevention and treatment of peripheral neuropathy, and with NSAID for the prevention and treatment of gastroduodenal ulcers.

The percentage of active ingredient in each pharmaceutical composition and the effective amount of the active ingredient used to practice the present invention for treatment of the disclosed conditions will be decided by the attending physician or veterinarian. Such amount of the compound as determined by the attending physician or veterinarian is referred to herein as the "effective amount". In general, a dosage range of the compounds in the composition is envisaged for administration in human medicine of from about 0.001 to 50 mg/kg of body weight daily. In the case of intravenous compositions, the dosage is for example about .1 to 0.6 mg/kg/day, and for oral compositions the dosage is about 0.5 to 10 mg/kg/day, more preferably 1.5 to 9 mg/kg/day.

Amounts of drug administered to produce serum levels 10-1000x the IC_{50} for inhibition of oligosaccharide processing in the L-PHA assay are preferably employed. It will also be appreciated that it may be necessary to deviate from the amounts mentioned and in particular to do so as a function of the body weight of the animal to be treated, the particular disease to be treated, the nature of the administration route and the therapy desired. In addition, the type of animal and its individual behaviour towards the medicine or the nature of its formulation and the time or interval at which it is administered may also indicate use of amounts different from those mentioned. Thus it may suffice, in some cases, to manage with less than the above-mentioned minimum amounts whilst in other cases the upper limit mentioned must be exceeded. Where major amounts are administered, it may be advisable to divide these into several administrations over the course of the day.

A compound of the invention may be used as a vaccine adjuvant to induce a potent immune response to itself and/or induce immunity to antigens, particularly antigens that are normally poor immunogens. A compound of the invention may augment vaccine immunogenicity

through activation of antigen presenting cells, such as monocytes or macrophages, to release cytokines that can promote T-cell help for B cell and CTL response. As a result, the compound may induce a more favorable antibody response with high titers, which appear earlier in the course of immunization and persist over time, as well as increase memory responses and CD8+ MHC Class I-restricted CTL. A compound of the invention may be contained in a vaccine or it may be administered separately. A compound of the invention may be used to enhance immunogenicity of antigens that induce T cell responses (e.g. T cell antigens), and in particular they may be used to enhance the immunogenicity of carbohydrate antigens associated with cancers or infectious diseases. Examples of vaccines which may employ a compound of the invention to augment immunogenicity include cancer vaccines (e.g. breast cancer vaccines), and vaccines for chronic infectious diseases.

The following non-limiting examples are illustrative of the present invention:

Example 1

Synthesis of (1*S*, 2*R*, 8*R*, 8a*R*)-8-Methoxymethoxy-1, 2-(isopropylidenedioxy)indolizidine (2). To a stirred solution of swainsonine acetonide **1** (107 mg, 0.50 mmol) in dry THF (2.5 mL) was added sodium hydride (60% in mineral oil; 40 mg, 1.0 mmol) and the mixture was stirred for 20 min. Tetrabutylammonium iodide (37 mg, 0.10 mmol) and chloromethyl methyl ether (76 L, 1 . 0 mmol) were added. The reaction mixture was heated at reflux for 16 h. TLC showed the reaction was complete. The reaction mixture was concentrated and the residue was purified by column chromatography (hexane/EtOAc = 1:1), giving 109 mg (84%) of the title compound as a pale yellow oil. ^{1}H NMR ($CDCl_3$, ref TMS, 500 MHz) δ: 4.84 (d, J = 6.5 Hz, 1 H), 4.69 (d, J = 6.5 Hz, 1 H), 4.63 (dd, J = 6.2, 4.7 Hz, 1 H), 4.58 (dd, J = 6.2, 4.0 Hz, 1 H), 3.76 (ddd, J = 10.9, 9.4, 4.7 Hz, 1 H), 3.40 (s, 3 H), 3.13 (d, J = 10.7 Hz, 1 H), 2.97 (br d, J = 10.5 Hz, 1 H), 2.16 (m, 1 H), 2.10 (dd, J = 10.7, 4.3 Hz, 1 H), 1.83 (td, J = 11.0, 3.4 Hz, 1 H), 1.59-1.70 (m, 3 H), 1.47 (s, 3 H), 1.31 (s, 3 H), 1.18-1.26 (m, 1 H); MS (CI, CH_4) *m/z*: 258 (M+H), 242, 228, 196.

Synthesis of (1*S*, 2*R*, 8*R*, 8a*R*)-8-Methoxymethoxy-1, 2-(isopropylidenedioxy)indolizidine *N*-oxide (3). To a stirred solution of **2** (240 mg, 0.93 mmol) in dichloromethane-ethanol (1:1, 1 mL) was added hydrogen peroxide (30%, 0.4 mL). The mixture was heated at 65°C (bath temperature) for 14.5 h. TLC showed the reaction was complete. 10% Palladium on carbon (16 mg) was added and the mixture was stirred at room temperature for 2.5 h. The mixture was filtered and the filtrate was dried twice over anhydrous sodium sulfate, filtered, and concentrated. The residue was dried *in vacuo* overnight, giving 167 mg of the title compound as a pale yellow syrup, which was used in the next step without further purification.

Synthesis of (1*S*, 2*R*, 8*R*, 8a*R*)-8-Methoxymethoxy-8a-methyl-1, 2-(isopropylidenedioxy)-indolizidine (4). To a stirred solution of the crude *N*-oxide **3** (167 mg, 0.61 mmol) in dry dichloromethane (1.5 mL) at −10°C was added trifluoroacetic anhydride (0.17 ml, 1.22 mmol) over 20 min. The mixture was stirred at 0°C for 2.5 h and then at room temperature for 0.5 h. The

reaction mixture was concentrated. The residue was dried *in vacuo* for 0.5 h and dissolved in dry THF (4 mL). To the resulting solution was added methylmagnesium bromide (3.0 *M* solution in diethyl ether; 0.82 mL, 2.4 mmol) at -78°C over 10 min. Stirring was continued at –78°C for 2.5 h. Water (10 mL) was added and the mixture was warmed to room temperature. The aqueous layer was extracted with ethyl acetate (4x10 mL). The combined organic layers were dried over anhydrous sodium sulfate and concentrated. The residue was purified by column chromatography (CH_2Cl_2:MeOH = 20:1), giving 104 mg (41%, three steps) of the title compound as a yellow oil. ^{1}H NMR ($CDCl_3$, ref TMS, 500 MHz) δ: 4.84 (d,J=6.4 Hz, 1 H), 4.65 (d, J = 6.6 Hz, 1 H), 4.61 (dd, J = 5.6, 5.1 Hz, 1 H), 4.27 (d, J = 6.0 Hz, 1 H), 3.83 (dd, J = 11.5, 4.7 Hz, 1 H), 3.39 (s, 3 H), 2.90 (d, J = 10.4 Hz, 1 H), 2.60-2.67 (m, 2 H), 2.33 (m, 1 H), 1.89 (m, 1 H), 1.60 (m, 2 H), 1.50 (s, 3 H), 1.40-1.47 (m, 1 H), 1.30 (s, 3 H), 0.77 (s, 3 H); MS (ES) *m/z:* 272(M+H), 210.

Synthesis of (1*S*, 2*R*, 8*R*, 8a*R*)-8a-Methyl-1, 2, 8-trihydroxyindolizidine (5). To a stirred solution of **4** (97 mg, 0.36 mmol) in THF (4 mL) was added 6 *N* HCl (4 mL). Stirring was continued for 63.5 h. The reaction mixture was concentrated under reduced pressure. The residue was dried *in vacuo*, diluted with methanol (2 mL), neutralized with concentrated ammonium hydroxide and filtered. The crude product was purified by preparative HPLC, giving 35.4 mg (54%) of the title compound as a white crystalline solid. ^{1}H NMR (D_2O, ref HDO, 500 MHz) δ: 4.29 (ddd, $J_{2,1}$ = 6.4 Hz, $J_{2,3}$ = 3.5 Hz, $J_{2,3}$ = 8.0 Hz, H-2), 3.91 (dd, $J_{8,7eq}$ = 4.8 Hz, $J_{8,7ax}$ = 11.8 Hz, H-8), 3.71 (d, $J_{1,2}$ = 6.4 Hz, H-1), 2.89 (dd, $J_{3\ ,2}$ = 8.0 Hz, $J_{3\ ,3}$ = 11.2 Hz, H-3), 2.67 (dd, $J_{3\ ,2}$ = 3.5 Hz, $J_{3\ ,3}$ = 11.2 Hz, H-3), 2.43 (m,$J_{5eq,5ax}$ = 12.5 Hz, $J_{5eq,6ax}$ = 5.5 Hz, $J_{5eq,6eq}$ = 2.0 Hz, H-5eq), 2.39 (m, $J_{5ax,5eq}$ = 12.5 Hz, $J_{5ax,6ax}$ = 12.2 Hz, $J_{5ax,6eq}$ = 3.6 Hz, H-5ax), 1.63 (m, $J_{7eq,6ax}$ = 4.7 Hz, $J_{7eq,6eq}$ = 2.5 Hz, $J_{7eq,7ax}$ =12.6 Hz, H-7eq), 1.44-1.57 (m, H-6ax & H-6eq), 1.39 (m, $J_{7ax,6ax}$ = 12.6 Hz, $J_{7ax,6eq}$ = 4.7 Hz, $J_{7ax,7eq}$ = 12.6 Hz, H-7ax), 0.83 (s, CH_3); ^{13}C NMR (D_2O, ref external 1, 4-dioxane, 125 MHz) δ 76.4 (C-1), 68.1 (C-2), 67.9 (C-8), 65.3 (C-8a), 55.8 (C-3), 42.6 (C-5), 26.8 (C-7), 21.1 (C-6), 9.3 (CH_3); MS (ES) *m/z:* 210 (M+Na), 188 (M+H), 170, 152.

Example 2

Inhibition of Golgi α-mannosidase II and Lysosomal α-mannosidase

The test compound, (1S,2R,8R,8aR)-8a-methyl-1,2,8-trihydroxyindolizidine, is prepared by 0.4 serial dilution of a 40 μM stock. Present in each determination is 10 μl diluted test compound, 25 μl of 10 mM paranitrophenyl mannopyranoside, 200 mM sodium acetate, pH 5.6 and 15 μl of purified rat liver Golgi mannosidase II. After incubating the reaction for 60 minutes at 37°C, the reaction is quenched with 50 μl of 0.5M sodium carbonate. Absorption is read at 405 nm. After subtracting the blank from positive controls and samples, the samples are normalized against the positive control mean using a variable slope, sigmoidal curve fit, with bottom = 0, top = 100. The signal is proportional to the amount of products from the uninhibited reaction. The calculated IC_{50} for inhibition of purified Golgi mannosidase II by the test compound is 18.214 ±

4.437 μM.

The effects of the test compound on lysosomal mannosidase were measured by adding (10μl) of the compound into 96 well Elisa plates followed by the addition of 200 mM sodium acetate pH 5.0 and 25 μl of 10 mM p-nitrophenyl α-D-mannospyranoside. 15 μl of lysosomal mannosidase (about 8mM/mL) was added to each well and the plates were incubated for 60 min at 37°C. The reaction was stopped by the addition of 50 μl of 0.5M sodium carbonate and formation of p-nitrophenol was measured with a plate set at 405. The calculated IC_{50} for inhibition of lysosomal mannosidase by swainsonine hydrochloride is 9.388 ± 3.021 μM.

Example 3

Representative *In Vivo* and *In Vitro* Protocols for Testing Compounds of the Invention

A. Administration of Test Compound for the Inhibition of Lung Metastasis

B16F10 melanoma tumor cells are cultured for 48 hours in the presence or absence of the test compound (0.36 μg/ml) before injection of 10^5 cells into the lateral tail veins of C57BL mice. Lung nodules are counted on day 24 after injection of tumor cells as described in Dennis, JW, Cancer Res. 46:5131-5136, 1986.

B. Inhibition of tumor cell colonization of the lung

Mice are given drinking water with or without 5.0 μg/ml of the test compound 2 days before tumor cells are injected into the lateral tail vein and maintained on the test compound for periods of 1-17 days. Lung nodules are counted on day 24 after injection of tumor cells.

C. Inhibition of human tumor growth in mice

Athymic nude mice injected subcutaneously with MeWo, a human melanoma tumor cell line, are treated with once daily ip injections of sterile saline or 20 μg/mouse of test compound in sterile saline. Tumor size is measured twice weekly with calipers and tumor weights are measured 4 weeks after tumor cell injection as per the method of Dennis, JW (Cancer Res. 50:1867-1872, 1990).

D. Determining Synergy of a Test Compound with the interferon-inducing agent Poly (I.C.) for inhibition of solid tumor growth

Mice are provided with drinking water either with or without test compound (3.0 μg/ml) 2 days before 10^5 MDAY-D2 tumor cells are injected. Tumor diameters are measured with calipers twice weekly, then on day 15 after tumor cell injection, tumors are excised and weighed. The tumor growth rate and tumor weight on day 15 in mice given test compound supplemented drinking water and/or two i.p. injections of poly (I.C.) are compared as described in Dennis JW Cancer Res. 46:5131-5136, 1986.

E. Enhancement of the Anti-proliferative effect of Interferon in vitro

HT29m, SN12C11 human carcinoma cells or MeWo melanoma cells are seeded into 5% FBS in MEM tissue culture medium at 10^3/ml in the presence and absence of a test compound (approximately 1.2μg/ml) either with or without 1000 IU/ml of human interferon alpha-2 (intronA,

Schering-Plough). The cells are cultured at 37°C in a 5% CO_2 atmosphere and on day 5 the cell number is determined. The method is as described by Dennis, J.W. JNCI 81:1028-1033, 1989.

F. In Vitro Progenitor Cell Assay

At specified times after treatment with between 0.7 and 5.0 μg/ml of test compound, control, and treated mice are killed by cervical dislocation. Bone marrow (BM) and spleen cells from each are processed according to the procedures of the GIBCO-BRL Mouse Bone Marrow Stem Cell Proliferation Kit (Cat. # 3827SA, Grand Island, NY). The potential colonies that form in the semi-solid medium are the CFU-GEMM, the CFU-GM, and the BFUs. The plates are incubated for 10-14 days at 37°C in a humidified atmosphere of 5% CO_2 and 95% air, and colonies consisting of at least 40 cells are enumerated using an inverted microscope (20X magnification) to demonstrate stimulation of hematopoietic progenitor cell growth.

G. Bone Marrow Proliferation Assay

Mice are treated with either 3 μg/ml of test compound in their drinking water or injected with 20 μg/mouse of test compound daily for 2-6 days. Proliferation is assessed by the incorporation of [^{3}H]-thymidine (5 μCi/ml) for 18 hours at 37°C into cultures containing equal numbers of freshly isolated BM cells in complete medium. The radiolabeled cells are collected with the aid of a cell harvester onto glass filters, and radioactivity is determined using a liquid scintillation counter. Cellularity of the bone marrow is also determined by using the Coulter counter to directly count BM cells after they are flushed from the tibias and femurs.

H. In vivo progenitor assay: Spleen Colony Formation Assay

Mice (10-14 weeks old) are x-irradiated for a total whole body exposure of 700cGY. The irradiated mice are maintained on sterile drinking water (approximately 3 μg/ml) and are given antibiotics to minimize mortality from infection. The number of BM stem cells is estimated by the method of Till and McCulloch (Biochim Biophys Acta 1980 Nov 26;605(4):431-59), which is based on the ability of intravenously injected progenitor stem cells to form colonies in the spleens of recipient mice previously exposed to a lethal dose of whole-body irradiation. The number of CFUs is proportional to the number of pluripotent hematopoietic stem cells present in the hematopoietic graft. Ten days after transplantation, recipient mice are sacrificed, their spleens are removed and fixed in Bouin's solution, and grossly visible colonies are counted.

I. Bone marrow transplant and repopulation

Prior to transplantation with bone marrow cells, mice are pre-treated with either a lethal dose of a chemotherapeutic agent or a lethal dose of x-irradiation, as described in White et al (Cancer Communications 3:83, 1991) and Oredipe et al. (JNCI 83:1149, 1991). Mice aged 10-14 weeks, are irradiated using Phillips RT 250 x-ray machines (two opposing therapeutic 250 Kvp x-ray machines, 235 KV, 15 mA, filtration 0.25 copper and 0.55 aluminum, with a half layer of 0.99 mm copper). Irradiation occurs with a dose rate of 126 cGy/min (63 cGy/min X 2) for 5 minutes and 33 seconds, for a total whole body exposure of 700 cGy. This level of irradiation exposure is

within the range described as being lethal for mice. After x-irradiation, animals are infused with 10^5 bone marrow cells freshly prepared from either control or test compound-treated donor mice. The test compound-treated donor mice receive approximately 20 μg/ml of test compound for 6 days. Recipient mice are monitored for survival over a period of 30 to 50 days.

J. Th1 immune response: Natural Killer (NK) and lymphokine-activated killer (LAK) cell assays

Human peripheral blood mononuclear cells (PBMCs) are isolated from whole blood using standard methods (Rees et al; J. Immunol Meths., 62:79-85, 1983; or Sedman et al, Br. J. Surg. 75: 976-981, 1988). The PBMCs are seeded into six-well plates in 5 ml cultures at a concentration of 1.5 million cells per ml either alone (control) or with varying concentrations of test compound, together with 1000 International Units (IU)/ml of IL-2 for three days for the LAK assay or 1000 IU/ml interferon-alpha overnight for the NK assay. The NK cell activity of the cultured PBMCs is measured in a Cr^{51} release assay using the K562 cell line (NK cell-sensitive) as target cells. LAK cell activity is measured using Cr^{51}-labeled Daudi cell line (NK cell-resistant) as targets.

K. Activity in mouse models of hepatitis

Drug activity against viral hepatitis may be determined by infecting mouse strains with mouse hepatitis virus-3 (MHV-3). Previous studies with MHV-3 have focused on mouse strains which develop fulminant hepatitis (Balb\cJ) or display resistance (A/J) to MHV-3 (Yuwaraj et al., 1996).

The CH3/HeJ strain, which develops chronic hepatitis in response to MHV-3 infection is treated with either saline or test compound (20 μg/mouse/day) alone or in combination with IFN. Before and during treatment, the levels and activation status of STATs is measured as well as serum cytokine levels, viral load and survival.

L. Activity in patients with chronic hepatitis C

The response to treatment with a test compound or test compound plus interferon-alpha in patients with chronic hepatitis C can be monitored by a decrease in viral load and serum liver alanine aminotransferase (ALT) measured during treatment, for example at 3, 6, and 12 months. Improvement in liver histology can also be assessed by performing biopsies before and after treatment.

The test compound is administered orally, twice daily, at doses between 50 and 200 μg/kg either alone, or in combination with alpha-interferon, which is administered at doses of 1 to 3 MU three times weekly. During this time, the test compound may be administered continuously or intermittently (e.g. 2 weeks on, one week off). The response in patients receiving test compound is compared to the response in patients receiving placebo or alpha-interferon.

Detection of hepatitis C viral RNA in serum, liver, and peripheral blood mononuclear cells is performed by the reverse transcriptase-polymerase chain reaction method (RT-PCR), using primer specific for the highly conserved, 5'-untranslated region (UTR) for qualitative or, with

appropriate internal control RNA, quantitative detection. The second method is a signal amplification or branched chain DNA (bDNA) assay. Viral nucleic acids are hybridized to microtiter plates and reacted with virus-specific extender probes followed by bDNA polymers.

For improvement in liver histology, the Histologic Activity Index based on a scoring system developed by Knodell et al (Hepatology 1981, 1:431-435), assigns grades in four categories: periportal necrosis, interlobular necrosis, portal inflammation and fibrosis. Alternatively, a system based on grading hepatic inflammation (0-4) and staging fibrosis (0-4) can be used (Scheuer PJ, J. Hepatol 1991; 13:372-374).

M. Hemorestoration/Chemoprotection

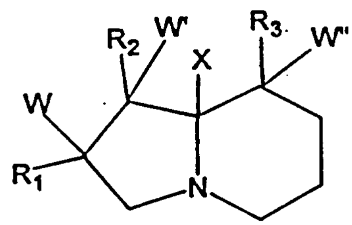
Cellular and animal models of hemorestoration/chemoprotection are described in Oredipe et al, 1991, supra, and White et al, 1991, supra.

While the present invention has been described with reference to what are presently considered to be the preferred examples, it is to be understood that the invention is not limited to the disclosed examples. To the contrary, the invention is intended to cover various modifications and equivalent arrangements included within the spirit and scope of the appended claims.

All publications, patents, and patent applications are herein incorporated by reference in their entirety to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety.

WE CLAIM:

1. A compound of the formula I



I

wherein

(a) R_1, R_2 and R_3 are each independently hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, or R_1 and R_2 together form a carbocyclic or heterocylic ring;

(b) W and W" and W' are each independently hydrogen, hydroxyl, alkoxy, thiol, thioalkyl, thioaryl, halo or amino, or W and W' together form a carbocyclic or heterocyclic ring; and

(c) X represents alkyl, alkenyl, alkynyl, cycloalkyl, alkoxy, or aryl,

and salts and optically active and racemic forms of a compound of the formula I.

2. A compound of the formula I as claimed in claim 1 wherein R_1, R_2, and R_3 represent hydrogen.

3. A compound of the formula I as claimed in claim 1or 2 wherein W, W', and W" represent hydroxyl.

4. A compound of the formula I as claimed in claim 1 or 3 wherein R_1, R_2, and R_3 are each independently alkyl, alkenyl, alkynyl, or aryl.

5. A compound of the formula I as claimed in claim 1or 3 wherein R_1, R_2, and R_3 are each independently alkyl or aryl.

6. A compound of the formula I as claimed in claim 1or 3 wherein R_1 and R_2 together form a carbocyclic or heterocyclic ring.

7. A compound of the formula I as claimed in claim 1, 2, 4, or 5 wherein W, W', and W" represent hydroxyl, alkoxy, thiol, thioalkyl, thioaryl, halo, or amino.

8. A compound of the formula I as claimed in claim 1, 2, 4, or 5 wherein W and W' together form a carbocyclic or heterocyclic ring.

9. A compound of the formula I as claimed in any one of claims 1 to 8 wherein X represents alkyl, alkenyl, alkynyl, aryl, cycloalkyl or alkoxy.

10. A compound of the formula I as claimed in any one of claims 1 to 8 wherein X

represents alkyl.

11. A compound of the formula I as claimed in claim 1wherein R_1, R_2, and R_3 represent hydrogen, W, W', and W" represent hydroxyl, and X represents methyl, ethyl, phenyl, benzyl, or methoxy.

12. A compound of the formula I as claimed in claim 1 which is (1*S*, 2*R*, 8*R*, 8a*R*)-8a-methyl-1,2,8-trihydroxyindolizidine, (1*S*, 2*R*,8*R*, 8a*R*)-8a-ethyl-1,2,8-trihydroxy-indolizidine, (1*S*, 2*R*, 8*R*, 8a*R*)-8a-propyl-1, 2, 8-trihydroxyindolizidine, or (1*S*, 2*R*, 8*R*, 8a*R*)-8a-butyl-1, 2, 8-trihydroxyindolizidine.

13. A process for preparing a compound of the formula I as claimed in claim 10 comprising (a) protecting swainsonine acetonide at the 8 position with a protecting group; (b) converting the protected swainsonine acetonide to an N-oxide; (c) reacting the N-oxide with trifluoroacetic anhydride; (d) reacting the acylated N-oxide with a nucleophile under basic conditions; and removing the protecting groups.

14. A pharmaceutical composition comprising a compound of the formula I as claimed in any one of claims 1 to 12 as an active agent, and a pharmaceutically acceptable carrier, excipient or diluent.

15. A method for stimulating the immune system, treating proliferative disorders, or microbial or parasitic infections in a patient comprising administering an effective amount of a compound of the formula I as claimed in any one of claims 1 to 12.

16. Use of a compound of the formula I as claimed in any one of claims 1 to 12 in the preparation of a medicament for stimulating the immune system, treating proliferative disorders, or microbial or parasitic infections.

17. A method for the treatment of cancer comprising administering to a subject an effective amount of a compound as claimed in any one of claims 1 to 12.

18. A method as claimed in claim 17 wherein the treatment comprises inhibiting metastasis or neoplastic growth.

19. A method for stimulating hematopoietic progenitor cell growth comprising administering to a patient an effective amount of a compound as claimed in any one of claims 1 to 12.

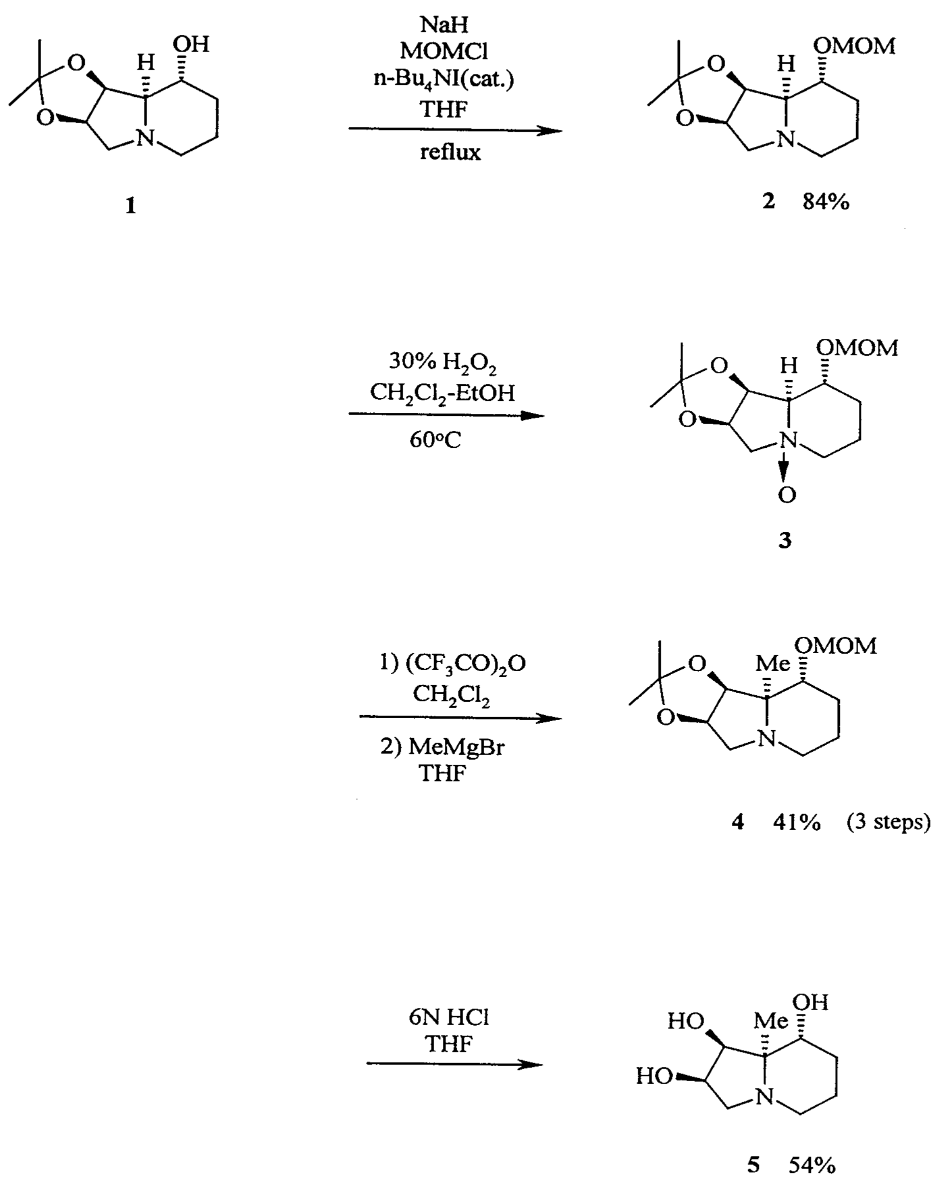
20. A method as claimed in claim 19 wherein the patient has been administered a myelosuppressive agent or is a bone marrow transplant recipient.

21. A method for treating a viral, bacterial, fungal, or parasitic infection in a patient comprising administering to a subject an effective amount of a compound as claimed in any one of claims 1 to 12.

22. A method of treating hepatitis C comprising administering to a patient an effective amount of a compound as claimed in any one of claims 1 to 12.

Figure 1

Synthesis of (1S, 2R, 8R, 8aR)-8a-Methyl-1, 2, 8-trihydroxyindolizidine



* MOM = CH_3OCH_2-